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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/675,113	09/30/2003	Latha Sundar	25436/1715	4984

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EXAMINER	
MARVICH, MARIA	
ART UNIT	**PAPER NUMBER**
1636	

DATE MAILED: 01/03/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/675,113	SUNDAR, LATHA	
	Examiner	Art Unit	
	Maria B Marvich, PhD	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-51* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-51* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *30 September 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *9/28/04, 8/26/04,*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☒ Other: *IDS 11/26/03*.

DETAILED ACTION

Claims 1-51 are pending in this application.

Information Disclosure Statement

Information Disclosure Statements filed 11/26/03, 8/26/04 and 9/28/04 have been identified and the documents considered. The signed and initialed PTO Form 1449s have been mailed with this action. The International Search Report, document 12, on the IDS filed 11/26/03, the International Preliminary Report on the IDS filed 8/26/04 and the International Search Report, document 2 on the IDS filed 9/28/04, have been considered. However these documents are not considered to be a published reference. Therefore, they have been crossed off of each PTO-1449.

Specification

For benefit claims under 35 U.S.C. 120, 121 or 365(c), the reference must include the status of nonprovisional parent application(s) (whether patented or abandoned) should also be included. If a parent application has become a patent, the expression "now Patent No. _____" should follow the filing date of the parent application. If a parent application has become abandoned, the expression "now abandoned" should follow the filing date of the parent application. In the instant case, the status of the parent applications should be updated in the first line of the specification.

The disclosure is objected to because of the following informalities: there is a large blank space on page 35, prior to the Examples section. It is not clear if there were text or tables on

these pages that have been omitted or if these are intentional blanks. Appropriate correction is required.

Priority

Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 120 as follows: An application claiming the benefit of a prior filed copending national or international application under 35 U.S.C. 120 must name as an inventor at least one inventor named in the prior filed application (see 35 USC 120). In the instant case, applicants claim benefit to commonly owned US application 09/894,806. However, there are no commonly named inventors for the '806 Application with the instant application. Therefore, priority under 35 USC 120 to US application 09/894,806 and provisional application 60/415,389 is denied and the priority of the instant application is the filing date of nonprovisional application 60/415,389, October 12, 2002.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-51 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of generating storage stable *E. coli* competent cells in which the cells are grown in hypertonic medium prior to treatment to become competent, does not reasonably provide enablement for a method for generation of storage stable competent cells in

which the competent cells are any prokaryotic cell in which the cells are grown in hypertonic medium prior to treatment to become competent. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation (*United States v. Telectronics, Inc.*, 8 USPQ2d 1217 (Fed. Cir. 1988)). Whether undue experimentation is required is not based on a single factor but is rather a conclusion reached by weighing many factors (See *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Inter, 1986) and In *re Wands*, 8USPQ2d 1400 (Fed. Cir. 1988); these factors include the following:

1) **Nature of invention**. The invention recites a method of generating storage stable cells in which bacterial cells are gown in hyperosmotic culture medium and treated to become competent. The competent cells are contacted with either non-reducing or reducing sugar and dried in the presence of non-reducing sugar. The method utilizes cell culture techniques.

2) **Scope of the invention**. The claims recite broadly that the cells are grown in hyperosmotic salt concentration which is defined in several claims as 100-350 mM above isoosmotic for the given cell. Furthermore, the cells are contacted with any solution comprising reducing or non-reducing sugar and then dried in the presence of non-reducing sugar.

3) **Number of working examples and guidance**. The specification teaches that cells are grown in LB, Psi broth, SOB medium, SOC medium, Terrific Broth, TY medium. Further, it is stated that the cells are inoculated into NaCl-fortified growth medium (see e.g. page 17-19).

However, applicants do not describe the osmolarity of these cells. *E.coli* cells are grown in LB medium supplemented with 370mM NaCl which is 200 mM higher than standard LB (see example 1).

Guidance for the generation of competent cells by a variety of methods is provided and is well known in the art for a variety of cell types (see 20). The methods are said to be enhanced by the addition of 10-25% non-reducing sugar or reducing sugars during competence induction. Furthermore, cell suspensions are dessicated in the presence of non-reducing sugars and the concentration of the sugars must be between 4% to 25%.

4) **State of Art**. Methods of making competent cells are well known in the high art. However, the growth of cells in hyperosmolarity is not that well known in the art. The art teaches that hyperosmolarity is different for each genus, species and strain of prokaryotic cell (see e.g. Tunnacliffe, col 7, line 30-46). Specifically, Tunnacliffe teaches that the total concentration of salt should be at least about 0.2 M to 0.5M and in the case of *E. coli* should not exceed 0.6 M. However, for prokaryotic cells such as *M Roseus* that are halophilic the hypertonic medium would be vastly different and would require determination of isoosmolarity for eventual determination of hyperosmotic conditions (see Tanaka et al).

5) **Unpredictability of the art**. It is unclear how the disclosed method should be altered if cells other than *E.coli* are used. The specification teaches specifically, that *E.coli* cells are grown in media supplemented with NaCl. For competence, cells are supplemented with 20% fructose or 20% sorbital. The example teaches that if no additional sugar is added to the competence medium, 20% fructose, a reducing sugar, is added prior to dessication. Therefore, it would appear that either reducing or non-reducing sugar is added to the cell but the additional

requirement for non-reducing sugar is unclear. Therefore, the requirements of the sugars in the method are unclear as the method is taught for *E. coli.* Furthermore, the nature of the growth media and the requirements of sugar for any bacterial cell other than *E. coli* are unknown.

6) **Summary**. The invention recites a methods for the generation of storage stable competent cells. The unpredictability of using the claimed invention with a variety of cells and the unpredictability of determining the proper growth culture and sugar concentration or requirement are high and therefore, it is unpredictable that any cell in any competence buffer can be made storage stable upon growing cells in hyperosmolarity.

In view of predictability of the art to which the invention pertains and the lack of established protocols and the inability to predict the hyperosmolarity of the media for each cell type: undue experimentation would be required to practice the claimed methods with reasonable expectation of success, absent a specific and detailed description in the specification. Given the above analysis of the factors which the courts have determined are critical in determining whether a claimed invention is enabled, it must be concluded that the skilled artisan would have had to have conducted undue unpredictable experimentation in order to practice the claimed invention.

Claims 1-51 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

Applicants claim a method of generating storage-stable competent cells that are dependent on a combination of critical elements including bacterial cell types, hyperosmotic conditions and, in some cases, oxygen scavengers. Applicants thus claim a broad genus of hyperosmotic salt conditions suitable for any bacterial cells.

Applicants claim a genus of oxygen scavengers.

The written description requirement for genus claims may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant identifying characteristics, i.e. structure or other physical and/or chemical properties, by functional characteristics coupled with known or disclosed correlations between function and structure, or by a combination of such characteristics sufficient to show that the applicant was in possession of the claimed genus.

The specification teaches that cells are grown in LB, Psi broth, SOB medium, SOC medium, Terrific Broth, TY medium. However, applicants do not describe the osmolarity of these cells. Further, it is stated that the cells are inoculated into NaCl-fortified growth medium (see e.g. page 17-19). *E.coli* cells are grown in LB medium supplemented with 370mM NaCl which is 200 mM higher than standard LB (see example 1). None of the hyperosmolaric conditions are provided except that for *E.coli*. Therefore, there is no clear description of the compositional (i.e. "structural") or functional characteristics required of a cell culture medium for the variety of cell types intended for use in the instant invention. Neither applicant nor the prior art provide a correlation between the osmolarity of cell media for *E.coli* and a variety of other cell types for storage stable competent cells. Given the large size of hyperosmolarity mediums and the inability to determine which will support cell growth for the generation of

storage stable competent cells, it is concluded that the invention must be empirically determined. In an unpredictable art, the disclosure of one species would not represent to the skilled artisan a representative number of species sufficient to show applicants were in possession of claimed genus.

The specification teaches that oxygen scavengers can be used during cell drying. A an example, applicants teach that oxygen scavengers or oxygen scrubbers are oxygen absorbing paper or sachets available from EMCO packaging company. However, applicants do not describe in sufficient detail the function of the oxygen scavengers such that the structural or functional characteristics required of the scavengers are known. Neither applicant nor the prior art teach the necessary requirements of oxygen scavengers such that one of skill in the art would be able to identify the proper oxygen scavenger for use in the instant invention. In an unpredictable art, the disclosure of one species would not represent to the skilled artisan a representative number of species sufficient to show applicants were in possession of claimed genus. In an unpredictable art, the disclosure of one species would not represent to the skilled artisan a representative number of species sufficient to show applicants were in possession of claimed genus.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-20, 29-34, 36, 37, 39, 40, 42-45 and 48-51 are rejected under 35 U.S.C. 103(a) as being unpatentable over Barnea et al (2002/0081565; see entire document) or Jessee et al (WO 98/35018; see entire document) in view of Tunnacliffe et al (US 6,468,782;see entire document).

Applicants claim a method of generating storage stable competent cells following growth in hyperosmotic salt.

Barnea et al teach a method for generating storage-stable competent cells that are also transformed with exogenous DNA. In example 1 and 2, *E.coli* cells are made competent in CB-I buffer comprised of $CaCl_2$ in the presence of sucrose or trehalose (see e.g. page 5, paragraph 0059). In example 3, the cells were stored at –70 °C, -20 °C, and 4°C for 28 days with at least $(10)^5$ transformants/μg DNA (see e.g. page 8, table 5). Cells to be transformed are rehydrated in buffer used for the transformation (transformation buffer) and exhibit at least $(10)^5$ transformants/μg DNA (see e.g. page 5,, paragraph 0057 and table 2).

Jessee et al teach a method of generating storage stable competent cells that comprises drying cells above freezing (to 10 °C) for at least 8 hours (see e.g. page 11, line 2-3) in a vacuum under non-atmospheric pressure (see e.g. page 10,line 23-25). The competent cells are preferably E. coli (see e.g. page 6, line 5-24) and are made competent in a buffer containing $CaCl_2$ (see e.g. page 8, line 11-14). The cells are storage stable for at least 45 days at temperatures ranging from 4 °C to –80 °C with a transformation efficiency of at least $1(10)^5$ (see e.g. page 11, line 6-20). Jessee et al. teach the use of glass forming cryoprotectants (and combinations of) that include carbohydrates such as trehalose, sucrose, sorbitol, PVP, ficoll and fructose as well as gelatin (see e.g. page 9, line 14-28). Also provided is a method for transforming nucleic acids utilizing the components of the invention following rehydration of the

cells in transformation buffer in which cells exhibit at least $1(10)^5$ transformants/mg DNA (see e.g. page 12, 1-20).

Neither Barnea et al nor Jessee et al teach that the cells are grown in hyperosmotic salt.

Tunnacliffe et al teach methods of cryoprotection in which intracellular trehalose is induced, cells are then suspended in a stabilizing solution and dried (see e.g. abstract). Trehalose is induced by growth in osmotic stress or oxygen deprivation growing cells in conditions of high osmolarity between 200 mOsmoles to 1.5 Osmoles of salts such as NaCl and for *E.coli* this is between 200 mM to 600 mM (see e.g. col 6, line 57-col 58, line 55). The cells are then mixed with a drying solution that comprises non-reducing sugar such as trehalose or sorbitol (see e.g. col 9, line 10-36). Cells are dried above freezing and under a vacuum (see e.g. col 10, line 27-42). A variety of containers are used for the cells such as vials or molded containers that avoid moisture. According to this method, the cells can be stored above 37 C for at least 6 weeks (see e.g. col 4, line 15-30).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to dry the competent cells taught by Barnea et al or Jessee et al with the method of preservation taught by Tunnacliffe et al because Barnea et al and Jessee et al teach that it is within the ordinary skill of the art to generate and dry competent cells for storage and because Tunnacliffe et al teach that it is within the ordinary skill of the art to grow cells for prolonged stability in hyperosmotic salt. One would have been motivated to do so in order to receive the expected benefit of induced intracellular trehalose which Tunnacliffe teaches creased viability and enhanced storage of cells (see e.g. Tunnacliffe, col 4, line 7-15) for the storage of competent cells for which prolonged storage at ambient temperatures is essential (see e.g. Barnea, paragraph

0008). Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 35 and 38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Barnea et al (2002/0081565; see entire document) or Jessee et al (WO 98/35018; see entire document) in view of Tunnacliffe et al (US 6,468,782;see entire document) further in view of Jin et al (US 5,733,774; see entire document).

Applicants claim a method of generating storage stable competent cells following growth in hyperosmotic salt. Storage involves limiting the exposure of the cells to oxygen such as by use of oxygen scavengers.

The teachings of Barnea and Jessee and Tunnacliffe et al are as above except:

Neither Barnea et al nor Jessee et al not Tunnacliffe et al teach that oxygen in the storage solution is limited.

Jin et al teach that an essential feature on the storage of bacteria is removal and limiting the oxygen. Jin et al teach that this can be accomplished by use of oxygen scavengers (see e.g. col 3, line 33-60 and col 7, line 8-17).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to dry the competent cells taught by Barnea et al or Jessee et al in view of Tunnacliffe et al because Barnea et al and Jessee et al and Tunnacliffe et al teach that it is within the ordinary skill of the art to generate and dry competent cells for storage after growing cells for prolonged stability in hyperosmotic salt and because Jin et al teach that it is within ordinary skill in the art

to remove oxygen from the storage system. One would have been motivated to do so in order to receive the expected benefit of meeting an essential element for long-term stabilization of bacteria. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria B Marvich, PhD whose telephone number is (571)-272-0774. The examiner can normally be reached on M-F (6:30-3:00).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Remy Yucel, PhD can be reached on (571)-272-0781. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Maria B Marvich, PhD
Examiner
Art Unit 1636

December 20, 2004

GERRY LEFFERS
PRIMARY EXAMINER